ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5391

Isozyme-based study on the differentiation and phylogeny of salmonids

Kenichi Numachi

Original title: Isozyme ni yoru sakeka gyorui no bunka to keito no kenkyu

In: Iden 38(1): 4-11. 1984

Original language: Japanese

Available from:
Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1988

17 typescript pages

Secretary of State / Secrétariat d'État

90-01548/

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU BUREAU DES TRADUCTIONS

LIBRARY IDENTIFICATION – FICHE SIGNALÉTIQUE

Translated from - Traduction de	Into - En
Japanese	English

Author - Auteur

Kenichi NUMACHI

Title in English or French - Titre anglais ou français

Isozyme-based study on the differentiation and phylogeny of salmonids

Title in foreign language (Transliterate foreign characters)
Titre en langue étrangère (Transcrire en caractères romains)

Isozyme ni yoru sakeka gyorui no bunka to keito no kenkyu

Reference in foreign language (Name of book or publication) in full, transliterate foreign characters.
Référence en langue étrangère (Nom du livre ou publication), au complet, transcrire en caractères romains.

Iden

Reference in English or French - Référence en anglais ou français

Genetics

Publisher - Editeur	DATE OF PUBLICATION / DATE DE PUBLICATION			Page Numbers in original / Numéros des pages dans l'original
	Year / Année	Volume	Issue No. / Numéro	4–11
Place of Publication / Lieu de publication	1984	38	1	Number of typed pages / Nombre de pages dactylographiées: 15

Requesting Department / Ministère-Client: DFO

Branch or Division / Direction ou Division: Scientific Publications

Person requesting / Demandé par: Heather Cameron

Your Number / Votre dossier nº:

Date of Request / Date de la demande: 88-05-09

Translation Bureau No. / Notre dossier nº: 3416088

Translator (Initials) / Traducteur (Initiales): GN

JUN JUIN - 3 1988

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Canada

SEC 5-111 (84-10)

Department of the Secretary of State of Canada | Secrétariat d'État du Canada

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU | BUREAU DES TRADUCTIONS

Client's No.–N° du client	Department – Ministère	Division/Branch – Division/Direction	City – Ville
	DFO	Scientific Publications	Ottawa
Bureau No.–N° du bureau	**Language – Langue**	**Translator (Initials) – Traducteur (Initiales)**	**JUN JUIN - 3 1988**
3416088	Japanese	GN	

Extra-special edition: Biology of salmon

Isozyme-based Study on the Differentiation and Phylogeny of Salmonids (4)

By Kenichi NUMACHI

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Isozyme studies combining gel electrophoresis and specific dyeing of enzymes have made clear the existence of genetic control and variation of enzyme molecules, and have furthermore rapidly developed population genetic research guided by such variations. The strong point of isozymes is that the gene type of an individual can be read off directly and simply from an electrophoretic image. By using isozymes as landmarks, genetic research on natural populations progressed in various animals in which even classical genetics had not developed because of difficulties in cross-breeding and raising. It was the same for the multitude of creatures distributed in the sea world such as the oceans and fresh waters.

Population genetics and evolutionary genetics used to target research on the question of population and species differentiation, and did not deal with taxonomic groups higher than species. It was the same with research utilizing enzymes; at first there was no theoretical basis nor method of dealing with taxonomic groups of a higher order. But in the latter half of the 1960s, the phylogenetic origin of multiple alleles involved with lactic dehydrogenase and such as well as their homogeny throughout the vertebrates started to become clear.

From around 1968, while we, who were doing genetic research on riverine populations of chum salmon, at the same time were analyzing rainbow trouts which could be obtained fresh regardless of the season, we had noticed that these two species, as far as the ones we had analyzed were concerned, had totally homologous gene loci only, with the genes thereabove having been replaced to a certain extent. Meanwhile, in 1972 Nei and Rogers published formulas on genetic distance and similarity which numerically indicate the extent to which the genes above the corresponding gene loci have been replaced between populations. This sort of background led us to grapple with phylogenetic research on salmonids.

Salmonids are difficult to classify even among fishes, and we were aware from the outset of the fact that taxonomic debates were endless and that there were various theories on evolution and phylogeny as well. However, our intent was not to make a new departure on the taxonomy of salmon or try to substantiate or counter-argue the existing taxonomical views from a different angle. Our major interest was to find out to what level among salmonids differentiation has occurred by the replacement of genes, and how wide the genetic distance is between them compared to that of other animals. To what extent the estimation of differentiation age based on such methods agrees with paleontological knowledge, and if drastically different whether conversely an examination of the estimated values of the important genetic parameters used as the premise could be required, or how close the results of this sort of research totally differing in the form and method adopted might agree with erstwhile taxonomical studies based primarily on morphology and such, these were the things which had piqued our interest and expectation.

1. Salmonids

While salmonids are distributed all over the northern part of the northern hemisphere including the polar region, the ones we analyzed were 14 species including a number of subspecies and populations obtainable in Japanese and North Pacific waters. These have been summarized in Table 1.

As shown here, salmonids are divided into the four genera of Oncorhynchus, Salmo, Hucho and Salvelinus. Salmonids are regarded, both in the West and the East, as the most tasty of fishes, and for the Japanese the genus Oncorhynchus, especially the standard Japanese salmon or what we call chum salmon here, is representative of the salmon. It is the species used for "aramaki" (rush-wrapped salted salmon) at New Year's. This genus is thought to have been differentiated in Japanese waters in relatively recent times and is distributed only in the Pacific. It includes the tasty and expensive sockeye salmon and chinook salmon and such. The term "salmon," used originally to indicate Salmo salar, the salmon for Europeans such as the English, has now become a term to indicate the Pacific salmon or genus Oncorhynchus. S. salar is merely called Atlantic salmon at times and nothing more. (5)

The species of the genus Oncorhynchus, while varying by species, spend a longer period of their lives at sea than other genera. By living in the vast ocean, they have a biomass which could never be hoped for by life in fresh waters alone,

Table 1. Salmonids in Japanese and North Pacific waters

サケ科魚類 Salmonidae	
サケ属 *Oncorhynchus* Suckley, 1860	
カラフトマス	*O. gorbuscha* (Pink salmon)
シロサケ	*O. keta* (Chum salmon or dog salmon)
ベニザケ	*O. nerka* var. *nerka* (Sockeye salmon)
ヒメマス	*O. nerka* var. *adonis* (Kokanee salmon)
ギンザケ	*O. kisutch* (Coho solmon)
マスノスケ	*O. tschawytscha* (Chinook salmon)
サクラマス	*O. masou* f. *masou* (Masu salmon)
ヤマメ	*O. masou* f. *ishikawae*
アマゴ	*O. rhodurus* f. *macrostomus*
ビワマス(アメノウオ)	*O. rhodurus* f. *rhodurus*
ニジマス属 *Salmo* Linne, 1758	
*ニジマス	*S. gairdnerii* f. *irideus* (Rainbow trout)
*スチールヘッド	*S. gairdnerii* f. *gairdnerii* (Steelhead trout)
*ブラウン・トラウト	*S. trutta* (Brown trout)
イトウ属	*Hucho* Günther, 1866
イトウ	*H. perryi* (Huchen)
イワナ属	*Salvelinus* Richardson, 1832
*カワマス	*S. fontinalis* (Brook trout)
オショロコマ	*S. malma* (Dolly Varden)
ミヤベイワナ	*S. malma miyabei*
アメマス	*S. leucomaenis leucomaenis*
イワナ	*S. leucomaenis pluvius*
ゴギ	*S. leucomaenis imbrius*
*レーク・トラウト	*S. namaycush* (Lake trout)

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

* Denotes transplanted species from foreign countries. However, steelheads are generally few.

and in the northern waters as much as 400,000 tons are caught annually. Of this close to 40% are pink salmon, about 30% are chum salmon, followed by sockeye salmon, coho salmon and chinook salmon in that order. In the rivers of the Tohoku region and Hokkaido, chum salmon go upstream. In the rivers of part of Hokkaido, pink salmon go upstream. All species spawn once in their lifetime, perishing after spawning. Their form is very similar, and especially during their pelagic life even experts have mistaken different species for the same, such that there are some which cannot be easily distinguished by amateurs. Names are also confusing sometimes, so touching upon this briefly, generally when we speak of "masu" (salmon-trout) we refer to Masu salmon, but when "masu" is mentioned governmentally or legally such as in the Japanese-Soviet Fisheries Treaty it refers to "Karafuto-masu" (pink salmon).

The species of the genus Salmo are all foreign species and are the ones being freshwater-propagated at various places. While there are also strains which migrate to sea like the steelhead, this genus is more freshwater-dependent, and can mature and spawn over and over remaining in fresh waters. Rather than referring to them as "masu" in "kana" (alphabetically) they are written in "kanji" (hieroglyphically) as "masu," and together with some of the foreign Salvelinus species, are called "trout" in English. Rainbow trouts are native to the North American Pacific coast, brown trouts to Europe, northwestern Asia and North Africa, and this genus is not distributed in Japan.

The genus Salvelinus is widely distributed in the northern part of the northern hemisphere. In English it is generally called "charr." In Japan when Salvelinus is mentioned, it evokes a cave-dweller-like mystical ring, but in the north it is distributed to close to the sea and there are sea-migrating types as well. Most widely distributed is the one called S. alpinus, distributed everywhere north of latitude 45° North on the Eurasian continent, American continent, Greenland and such encompassing the polar region. Overlapping in distribution in part with

this is the Dolly Varden, distributed further south in the northwestern part of the American continent and the Aleutians, Kamchatka, the Kuriles, Hokkaido, all along the continuation of Primorskaya. This S. malma, which has an elegant body colour with crimson spots scattered over a light brown ground, is called Dolly Varden after the beautiful girl wearing a pink flower-patterned dress who appears in C. Dickens' novel. The lake trout is native to the northern part of the North American continent, and is such a unique species that a separate genus Cristivomer was once established for it. Recently on Salvelinus a voluminous work by Balon was published, but on this denizen of the north there are still felt to remain genetically interesting research tasks to be done hereafter.

The huchen is referred to as a phantom fish by fishermen, and its distribution is restricted to Eurasia, there being said to be five species in the world. The Danube River huchen is sometimes called Danube salmon.

2. Genetic differentiation of riverine chum populations

Before getting into the question of the speciation of salmonids, let us look at the variation within a species and the genetic differentiation between populations with the chum salmon. In the rivers of Hokkaido and the Tohoku region, chum salmons swim upstream and spawn. While the strictness of the chum's homing to its original river is not clear in detail, it does mean that there is a genetically differentiated, independent population in each river in accordance with the strictness of homing. Upon analyzing the isozymes and examining the 30 gene loci involving these in chums ascending each river, there were five gene loci or approximately 17% where allelomorphs occurred with a gene frequency of 5% or more, and the mean heterozygosity was 0.033. While the variability of fishes could be thought to be at about the average level for vertebrates, the variability (6) of salmonids is about the same as the chum salmon and slightly on the low side.

Upon examining the chums which ascend the rivers of the Tohoku region and Hokkaido, the gene frequency of the Icd-A1, Ldh-A2, Mdh-B gene loci involved

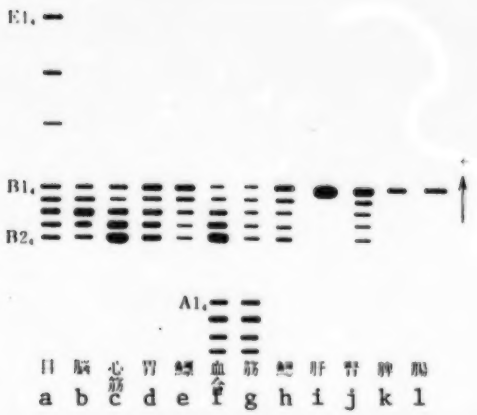
with isocitric dehydrogenase (ICD), lactic dehydrogenase (LDH), malic dehydrogenase (MDH) was found to differ variously depending on the river.

To indicate the genetic difference between populations various values can be used, but when the fixed index F_{ST} was determined by gene locus it was 0.004~0.062 with a mean of 0.018±0.0048. While this value is low compared to 0.055~0.081 obtained between human races with respect to the ABO blood groups, it is far larger than the 0.00065~0.00074 by prefecture. In addition, the coefficient of gene differentiation G_{ST} calculated from the mean heterozygosity was 0.043. While this value indicates that the majority of the variation of isozymes of the chum populations of Japan is within a population and the difference between populations is no more than 4.3% of the total variation, in terms of population-level differentiation it is not that small.

On the other hand, when the genetic distance according to Nei was determined, it came out to 0.0008-0.0192 with an average of 0.0055, indicating it to be roughly the same as the inter-population differentiation of drosophilae and such. Recently, OKAZAKI made a detailed analysis of the genetic composition throughout the entire distribution area of the chum salmon including the U.S., Canada and Soviet Union. As a result of that, he has pointed out that, with the chum salmon which has the shortest riverine life along with the pink salmon, the intermixture between riverine populations is somewhat high and there is genetic intermixing to some extent, and local populations consisting of a number of geographically adjacent riverine sub-populations similar in genetic composition are present throughout the distribution area of this species.

3. Differentiation of salmonids

Between chum populations there are variations in a number of gene loci, the compositions of which differed by population. But examining each of the salmonid species shown in Table 1, the gene loci involved with the enzymes each species has were all considered to be homologous. For example, Fig. 1 shows the



UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

a:	Eye	e:	Air bladder	i:	Liver
b:	Brain	f:	Blood-coloured flesh	j:	Kidney
c:	Myocardium	g:	Muscle	k:	Spleen
d:	Stomach	h:	Gill	l:	Intestine

Fig. 1. The LDH isozyme patterns $E1_4$, $B1_4$, $B2_4$, $A1_4$ of rainbow trout tissues show up as quadripartites of E1, B1, B2, A1 subunits respectively

isozyme pattern of LDH in rainbow trout, but every species indicated essentially the same pattern as this. Salmonids in comparison to the other species of the herring order are said to have approximately double the DNA content and chromosome number (Ohno, 1970), and the structural genes involved with enzymes are also almost doubled. With LDH the duplication of the E gene loci is not clear but the A and B genes are duplicated. In salmon the B1 and B2 gene loci occur in most tissues, and five B isozymes can be seen in which the B1 and B2 subunits are combined as quadripartites. However, in the liver, spleen and intestine, virtually just B1 occurs. The A gene locus occurs only in blood-coloured flesh and white muscle. The A subunit never combines with the B subunit as generally known in mammals. In the muscle the A1 and A2 subunits unite and become quadripartites, forming five A subunit isozymes. However, the occurrence of A2 is small in every tissue, so that the homotetramers of A2 are extremely few in number, and bunts are hard to see as well. The A and B isozymes are immunologically homologous to LDH-A and B in mammals, and the physicochemical properties are homologous as well.

In mammals the LDH-B isozyme which is preeminent in the myocardium always migrates electrophoretically more to the anode side than the LDH-A isozyme prevalent in skeletal muscle. In fish this does not always apply, so A and B cannot be identified by electrophoretic mobility. In LDH of salmon the magnitude of the electrophoretic mobility of B1 and B2 differs by species as in Fig. 2. That is, generally in the genus Oncorhynchus and genus Salmo the B1 isozyme migrates more to the anode side than B2, while among the cohorts of Masu salmon and in the genus Salvelinus B2 migrates more to the anode side than B1.

By clarifying the homogeny of such isozymes, the difference in the genes of the B1 gene locus for example is determined from the migrated position of the homopolymers of B1. Even if there be a replacement of the basic configuration of DNA or amino acids, not all of them will be electrophoretically detected. (8) Table 2 shows the difference in genes above the Ldh-B1 gene locus and such in terms of electrophoretic difference.

Looking at this table, the genes above Ldh-B1 for example show a variation in sockeye salmon and such, some changing to a and e, but in the genus Oncorhynchus,

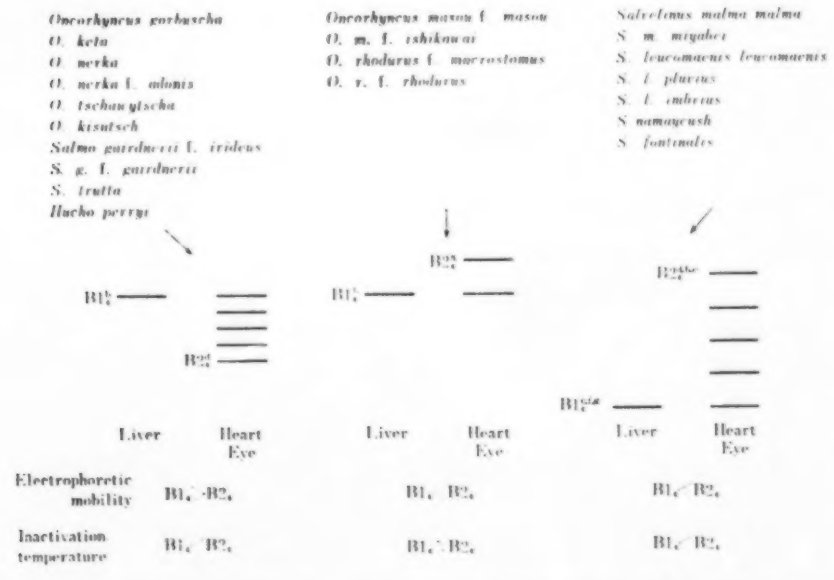


Fig. 2. Distribution and characteristics of LDH-B1 and B2 isozymes of salmonids

Table 2. Genes above the homologous gene loci of salmonids

第2表　サケ科魚類の相同遺伝子座上の遺伝子.

種 Species	*Ldh-B1*		*Ldh-B2*		*Ldh-A1*		*Mdh-B*	
	Monomorphic	Polymorphic	Monomorphic	Polymorphic	Monomorphic	Polymorphic	Monomorphic	Polymorphic
Oncorhynchus								
O. nerka f. nerka	—	a=.10, b=.90	d	—	b	—	—	e=.03, g=.97
O. n. f. adonis	b	—	d	—	b	—	g	—
O. keta	b	—	d	—	b	—	g	g=.98, b=.02
O. tschawytscha	b	—	d	—	b	—	g	—
O. kisutsch	b	—	d	—	b	—	g	—
O. gorbuscha	b	—	d	—	d	—	—	c=.02, g=.96, l=.02
O. masou f. masou	b	—	a	—	b	—	—	b=.83, f=.17
O. m. f. ishikawai	b	—	a	—	b	—	—	b=.90, g=.10
O. rhodurus f. macrostomus	b	—	a	—	b	—	—	a=.21, b=.79
O. r. f. rhodurus	b	—	a	—	b	—	—	a=.03, b=.94, g=.03
Salmo								
S. gairdnerii f. irideus	b	—	d	—	b	—	—	b=.88, g=.12
S. g. f. gairdnerii	—	b=.94, e=.06	d	—	b	—	—	b=.95, g=.05
S. trutta	b	—	d	—	b	—	b	—
Hucho								
H. perryi	b	—	b	—	a	—	b	—
Salvelinus								
S. malma malma	c	—	b	—	e	—	—	g=.94, h=.06
S. m. miyabei	c	—	b	—	e	—	—	c=.15, g=.85
S. leucomaenis leucomaenis	c	—	b	—	g	—	—	b=.32, g=.60, h=.08
S. l. pluvius (Nikko)	c	—	b	—	g	—	b	—
S. l. pluvius (Gifu)	c	—	b	—	g	—	—	b=.75, f=.25
S. l. imbrius	c	—	b	—	g	—	b	—
S. namaycush	c	—	b	—	f	—	k	—
S. fontinalis (Nijibetsu)	f	—	—	b=.20, c=.10, d=.70	e	—	—	c=.03, g=.97
S. fontinalis (Nikko)	f	—	—	b=.55, d=.45	e	—	—	c=.10, g=.90

genus Salmo and the huchen all are b, while in the genus Salvelinus, excepting the brook trout, all can be seen to have c. Looking at one gene locus, this many species of salmon can only be distinguished into the three of b, c, f. It can probably be viewed that the replacement of genes by mutation only progresses extremely slowly. Looking at the next gene locus Ldh-B2, within the genus Oncorhynchus for example, the cohorts of the Masu salmon differ from the others. By just looking at one or two gene loci like this, the species of salmonids are so similar that the species cannot be distinguished by the genes they possess, but by making a comparison of a series of gene loci, it can be seen that the genes have been replaced to varying degrees between species.

Rogers (1972) put forth the equation $S=1-\frac{1}{L}\sum_{i=1}^{L}[1/2\sum_{j=1}^{Ai}(Pijx-Pijy)^2]^{1/2}$ as a formula to express the degree of replacement of genes. It is called genetic similarity. L is the number of gene loci analyzed, Ai is the number of allelomorphs in the ith gene locus, and Pijx and Pijy are the frequencies of the jth allelomorphs possessed by species x and species y being compared. Table 3 shows the values of S determined in this way. If genetically they are totally similar S=1; if the genes have been changed to different ones in all gene loci S=0.

Meanwhile, Nei (1972) put forth genetic distance D as an index to express the degree of genetic replacement (see OHANE, 1977, etc.). He also estimated the differentiation age (t) as $t=D/(2cn_T\lambda a)$, where c is the proportion of the variation detectable by electrophoresis, n_T is the mean number of codons which determine the structure of proteins and λa is the mutation rate per nucleotide. Nei at first set c=0.4, n_T=800, $\lambda a=2.1\times10^{-9}$ and got $t=7.4\times10^{5}D$, but subsequently amended this and got $t=5\times10^{6}D$.

The data in Table 3 can be presented as a branching diagram like in Fig. 3. This diagram was obtained using D, incorporating D and t as scales. This diagram was only obtained from data on 13 gene loci, but it is felt to well illustrate

Table 3. Coefficient of genetic similarity of salmonids

第3表　サケ科魚類の遺伝的類似係数.

	Oncorhynchus gorbuscha	*O. keta*	*O. nerka* f. *nerka*	*O. n.* f. *adonis*	*O. tschawytscha*	*O. kisutsch*	*Salmo gairdnerii* f. *irideus*	*S. g.* f. *gairdnerii*	*Oncorhynchus masou* f. *masou*	*O. m.* f. *ishikawai*	*O. rhodurus* f. *macrostomus*	*O. r.* f. *rhodurus*	*Salmo trutta*	*Hucho perryi*	*Salvelinus fontinalis* (Nijibetsu)	*S. fontinalis* (Nikko)	*S. malma malma*	*S. m. miyabei*	*S. leucomaenis leucomaenis*	*S. l. pluvius* (Nikko)	*S. l. pluvius* (Gifu)	*S. l. imbrius*	*S. namaycush*
Oncorhynchus gorbuscha		.697	.683	.656	.592	.692	.522	.511	.405	.408	.407	.404	.498	.398	.233	.216	.197	.187	.168	.104	.115	.116	.104
O. keta			.786	.806	.706	.706	.630	.605	.514	.516	.522	.518	.506	.406	.238	.218	.200	.191	.170	.106	.116	.120	.106
O. nerka f. *nerka*				.984	.684	.681	.657	.645	.595	.597	.596	.578	.491	.493	.339	.320	.201	.193	.171	.108	.118	.121	.108
O. n. f. *adonis*					.700	.656	.665	.647	.608	.610	.609	.582	.500	.500	.332	.313	.194	.185	.163	.100	.110	.113	.100
O. tschawytscha						.900	.724	.712	.608	.610	.609	.606	.600	.500	.232	.213	.194	.185	.163	.100	.110	.113	.100
O. kisutsch							.724	.712	.608	.610	.609	.606	.600	.500	.232	.313	.194	.185	.163	.100	.110	.113	.100
Salmo gairdnerii f. *irideus*								.976	.741	.747	.739	.745	.700	.642	.200	.193	.127	.131	.160	.200	.201	.216	.121
S. g. f. *gairdnerii*									.826	.837	.723	.739	.702	.637	.191	.183	.122	.128	.157	.211	.193	.227	.119
Oncorhynchus masou f. *masou*										.985	.981	.962	.583	.583	.243	.235	.111	.115	.143	.183	.193	.196	.108
O. m. f. *ishikawai*											.982	.971	.590	.590	.245	.237	.113	.117	.146	.190	.178	.203	.105
O. rhodurus f. *macrostomus*												.961	.579	.579	.245	.236	.112	.116	.144	.179	.177	.193	.109
O. r. f. *rhodurus*													.597	.581	.226	.217	.117	.121	.150	.205	.187	.220	.113
Salmo trutta														.500	.036	.027	.003	.007	.036	.100	.075	.113	.100
Hucho perryi															.336	.327	.203	.207	.236	.300	.275	.313	.200
Salvelinus fontinalis (Nijibetsu)																.921	.441	.434	.311	.248	.258	.261	.248
S. fontinalis (Nikko)																	.515	.459	.332	.269	.279	.282	.269
S. malma malma																		.987	.867	.803	.813	.753	.603
S. m. miyabei																			.869	.807	.817	.757	.607
S. leucomaenis leucomaenis																				.936	.945	.886	.614
S. l. pluvius (Nikko)																					.975	.950	.600
S. l. pluvius (Gifu)																						.925	.610
S. l. imbrius																							.613
S. namaycush																							

.60～.80　.40～.60　.20～.40　<.20　<.80

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

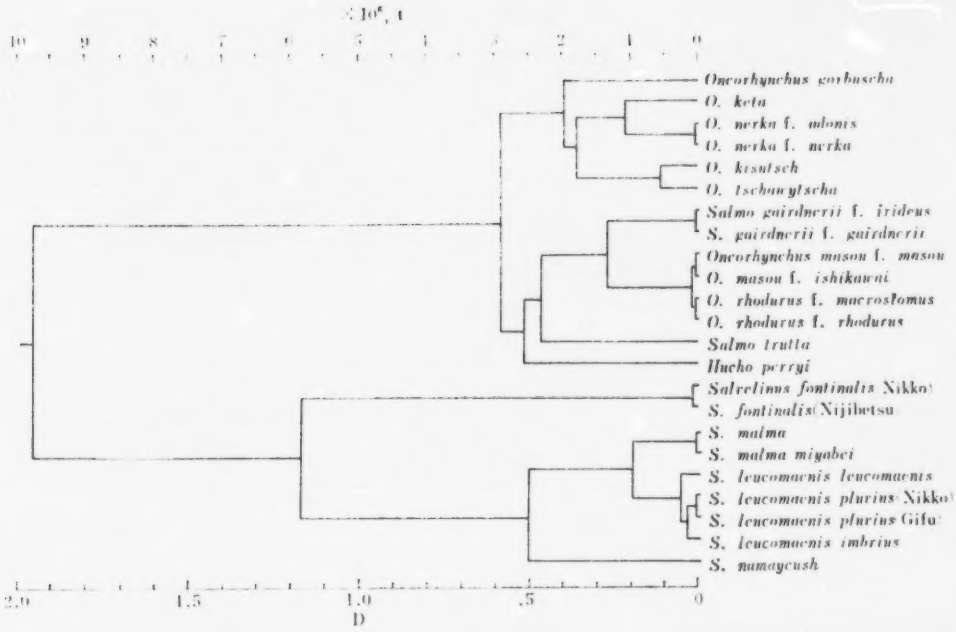


Fig. 3. Branching diagram based on the genetic distance of salmonids. Scale of differentiation age shown at top, genetic distance at bottom.

the affinitive relationship of salmonids. In this figure the salmonids broadly separate into the genus Salvelinus and the genus Oncorhynchus-genus Salmo group, with the Oncorhynchus-Salmo generic group dividing into the genus Oncorhynchus and genus Salmo. Also, the relationships of sockeye salmon and kokanee salmon, rainbow trout and steelhead trout, Masu salmon and O. masou f. ishikawae, Dolly Varden and S. malma miyabei and such closely related ones are shown in good agreement.

Rounsefell (1958, 1962) considered that among salmonids the Salvelinus types were primordial, and thought that ancestral forms of the Atlantic salmon and rainbow trouts were derived from an ancestral form of Salvelinus, while the Pacific salmons became differentiated from rainbow trouts. Combining this with the ideas of Neave (1958), it is being thought that first of all over two million years ago species of the Salvelinus type migrated to the Bering and Japan Sea area, subsequent to which one Glacial Period later a primordial species close to the (10)

Masu salmon migrated, which gave birth to the present-day genus Oncorhynchus in the Sea of Japan.

Of great interest in the branching diagram of Fig. 3 is the fact that cohorts of the Masu salmon are in the genus Salmo. The fact that the Masu salmon is, within the genus Oncorhynchus, closest to the genus Salmo not only in form but ecologically as well is something many researchers point out. This branching diagram agrees in places with the morphology-based theories of Hikita (1962) and such as well, and is also felt to be helpful concerning the classification groups in which many debates have occurred heretofore such as the interrelationship and distance between types of Masu salmon, the position of huchen, and the relationship and distance between Dolly Varden and Salvelinus types. On the other hand, however, as shown by the fact that the estimated value of the differentiation age is high if anything, in order to estimate the affinity of salmonids accurately, it goes without saying that there is a need to thoroughly examine many more gene loci. The results shown here are data limited to the initial stage, and do nothing more than show the way to approach such research.

4. Differentiation of fishes and isozymes

Salmonids, when looked at from the homogeny of their gene loci, can be thought to have been created by the doubling of nearly all the gene loci when an ancestral form close to Salvelinus became differentiated from another herring order. It can be thought that the differentiation of the family Salmonidae occurred by this doubling, while the species within the family Salmonidae developed by the genes above these gene loci being replaced by mutation. Of course, what is important in speciation, as seen in Table 2, is not the mutation of one individual or above one gene locus, but the factors and the mechanism by which these get to be maintained at some frequency in a population or species, and this indeed is the big issue in population genetics.

Be that as it may, the number of present-day species of fish runs as high as 15~17 thousand species comprising 40% strong of all vertebrates, and they include species variegated not only in form but in ecology and physiology as well. In the differentiation of these fish species, in addition to the variation of structural genes shown in salmonids, it is thought that the variation of regulatory genes which control their function played a big role. Among salmon it was shown that LDH-B is preeminent in most tissues, but in the flatfish, (11) flounder and scorpionfish families LDH-A is preeminent in many tissues including the myocardium. Between LDH-A and B is a difference in zymochemical property, and the differentiation of such regulatory genes is felt to be an important factor in changing the physiology and ecology and even the habitat. Isozyme studies do not have the accuracy like amino acid configurations of proteins such as cytochrome C undertaken before, but they have the advantage of enabling the analysis of many more gene loci easily and making possible the analysis of regulatory genes which code them as well.

On the other hand, however, in studies of birds and dolphins we have undertaken in recent years keying on isozymes, we have also noticed that the differentiation between species ends up being estimated fairly small. Accordingly, we are proceeding with DNA analysis of the mitochondria on these, and we feel the differentiation of fishes also, with more varied methods, will get more detailed and clear genetically as well.

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

References

1) Balon E. K. : Charrs, Salmonid Fishes of the Genus *Salvelinus*. Dr. W. Junk br Publishers, Hague, Netherlands (1980).

2) Neave, F. : The origin and speciation of *Oncorhynchus*. Trans. Roy, Soc. Can., **52**, Ser. 3. Sec. **5**, 25-39 (1958).

3) Nei, M. : Genetic distance between populations. Genet., **68**, S47 (1971).

4) Nei, M : Interspecific gene differences and evolutionary time estimated from electrophoretic diversity. Amer. Nat., **105**, 385-398 (1972).

5) 大羽 滋：集団の遺伝. up バイオロジーシリーズ, 19, pp. 164, 東京大学出版会 (1977).

6) 岡崎登志夫：シロサケ (*Oncorhynchus keta*) の集団構造に関する遺伝学的研究. 博士論文, 東京大学, pp. 170 (1981).

7) Rogers, J. W. : Measures of genetic similarity and genetic distance. Studies in Genetics VII. Univ. Texas Publ., No. 7213, 145-153 (1972).

8) Rounsefell, G. A. : Anadromy in North American Salmonidae. U. S. Fish and wildlife Serv., Fish. Bull., 58(131), 171-185 (1958).

9) Rounsefell. G. A. : Relationships among North American Salmonidae. U. S. Fish and Wildlife Serv., Fish. Bull., **62** (209), 235-270 (1962).

5) OHANE, Shigeru: Shudan no iden (Genetics of populations). UP Biology Series, 19, pp. 164, University of Tokyo Publishing Society (1977).

6) OKAZAKI, Toshio: Shirosake (Oncorhynchus keta) no shudan kozo ni kansuru idengaku-teki kenkyu (Genetic studies on the population structure of chum salmon (Oncorhynchus keta)). Doctoral thesis, University of Tokyo, pp. 170 (1981).

(Kenichi NUMACHI, Ozuchi Centre, Oceanographic Research Institute, Univ. of Tokyo)